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HBV and HEV Co-infections among Animal Handlers and Non-Animal Handlers in Osun State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IOO, IRG and OOO designed the study and wrote the protocol. Authors IRG and FAO managed the analyses of the study and performed the statistical analysis. Author IOO managed the literature searches and wrote the first draft of the manuscript. Author OOO supervised the whole study which, author IRG used as part of her M.Sc Dissertation in the Department of Medical Microbiology and Parasitology, Ladoke Akintola University of Technology, Ogbomoso, Nigeria. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Co-infection of hepatitis A, B, C, D, and E viruses may lead to severe morbidity and mortality. This study was conducted to determine prevalence of co-infection of HBV and HEV among animal and non-animal handlers in Osun State, Nigeria.
Study Design: Cross-sectional study.
Place and Duration of Study: Ladoke Akintola University of Technology (LAUTECH), Nigeria, between June 2015 and July 2019.
Methods: A total of 180 blood samples were obtained and screened for Hepatitis B and E virus from cohorts of 90 animal handlers and 90 non-animal handlers. Questionnaires on HBV and HEV were administered to obtain a demographic characteristic of the participants. HBsAg and anti-HEV antibodies were screened using HBsAg and HEV ELISA kits.
Results: Results showed the overall prevalence of HBV and HEV Co-infection to be 12.2 %. There was variation in the HBV/HEV co-infections rates among the studied population, with a co-infection

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rates of 15.9%, 14.3% and 7.8% for butchers, pig handlers and non-animal handlers, respectively. Sources of drinking water was the predisposition factor for HBV/HEV coinfections (P=0.02). The results revealed that subjects who used tap and river as a drinking water source had the highest prevalence followed by well and sachet and then all water source. Although results portray no statistically significant association with, frequent washing of hands after, rearing of animal, type of toilet, eating of pork, consumption of grilled meat and cow skin (P> 0.05).

Conclusion: This study reported a high prevalence of HBV/HEV coinfections among animal and non-animal handlers in Osun State, Nigeria. There is, therefore, the need to increase health promotion efforts such as immunization, health education, campaign, provision of adequate blood screening equipment and proper hygiene is recommended for further reduction in HBV/HEV transmission. Since the consumption of contaminated water is the main transmission route of HEV, improving the level of public health sanitation in the area should be considered a priority by policymakers.

Keywords: Co-infections; HBV; HEV; animal handlers; non-animal handlers; Nigeria.

1. INTRODUCTION

Hepatitis viruses are circulating worldwide, but their distribution patterns are different in every individual country [1]. Despite the existence of effective vaccines against hepatitis A and B, viral hepatitis is still a major global public health concern [1-3]. Hepatitis B virus (HBV) is a global life-threatening disease with hundreds of millions of individuals chronically infected. At least one fourth to one-third of the world's population had been infected with the HBV and about 400 million people are infected chronically [4]. In Nigeria, the prevalence of HBV chronic infection is 13.6% [5].

The disease caused by hepatitis E virus (HEV) infection is a major public health problem in Africa, especially in resource limited countries [6]. About 20 million cases and 3.3 million acute cases of HEV occur globally [7-9]. In the developed countries, genetic similarities between human HEV strains and those isolated from pigs, cows, chickens, rabbits, rats, and fish have been observed [10-12]. In African countries, a number of HEV outbreaks were reported in Ethiopia, Somalia, Uganda, Democratic republic of Congo, Sudan, South Sudan, and Nigeria [13-17].

Hepatitis E virus (HEV) like Hepatitis A virus (HAV) is mainly *transmitted* via the fecal-*oral* route especially through contaminated water and it is associated with large water-borne outbreaks [1]. Hepatitis E virus (HEV) is an enteric virus which could be transmitted through faecal-oral route [18]. Although HAV and HEV transmission routes are similar, their epidemiology is substantially different [1]. HEV can also be transmitted parenterally by blood transfusion or direct contact with infected animals [1,19-21]. The infection is self-limiting with mortality rate of about 1.0 to 2.0% in the general population. However, the mortality rate can increase up to 45.0% in high risk populations such as pregnant women [1,22]. In Immunodeficient or immunocompromised patients HEV infection may result in chronic infections. A meta-analysis study among Iranians reported an HEV seroprevalence of about 10.0% [1,23]. However, this prevalence increased up to 25.5% in high density populated areas of metropolitan cities of Iran [1,24].

Co-infection of HBV with other viral infections has been reported by several researchers [25-26]. Studies on co-infection of HBV and other viruses on different group has been carried out. Hepatitis B co-infection with HCV and HIV are known to affect the progression therapy management and clinical outcome of these infections [26], also the co-infection of tuberculosis (TB) and viral hepatitis infections in the same patient poses a unique challenge to such a patient [26]. Hepatitis B co-infection with malaria also leads to a public health challenges as reported by Omalu *et al*. [27]. But less information was available on HBV and HEV co-infection especially among animal handlers. Although, elimination of HBV is still a challenge, though vaccination and effective management strategies appear to be a progress towards achieving a success in the elimination of the virus [28].

Butchers and pig farmers are subjected to knife-cut, sharing of sharp object and so on with their colleagues which are among the risk factors of transmission of HBV [29,30]. Due to the abundance of asymptomatic or unreported cases, prevalence of such diseases is underestimated even under the best surveillance systems [1]. Therefore, epidemiological studies in different

geographical regions and among different population groups seem to be necessary to reveal the real prevalence and to estimate their true burden [1]. Thus, an urgent need for a study of this nature to provide necessary information, especially in this population. The aim of this study is to determine the prevalence of HBV and HEV co-infection among animal and non-animal handlers. Such data can help to develop appropriate prevention, vaccination and treatment programs.

2. MATERIALS AND METHODS

2.1 Study Area

This study was carried out among butchers, pig handlers and the non-animal handlers in Ife, Sekona and Osogbo, Osun State, South-Western Nigeria. The laboratory analysis was carried out in the Molecular Biology Laboratory, Ladoke Akintola University of Technology, Isale Osun, Osogbo, Nigeria.

2.2 Study Design

This is a cross-sectional study involving people whose occupation increased their risk of infection (both males and females). Ethical approval was obtained by the Osun State Ministry of Health, Osogbo. The protocol number was OSHREC/PRS/569T/3.

2.3 Study Population

The study participants comprised of people whose occupation increased their risk of infection (both males and females). The study participants included 90 animal handlers (69 cow butchers, and 21 pig handlers) and 90 non-animal handlers, making a total of 180 volunteers.

2.4 Sample Collection

Five (5 ml) of venous blood was collected from each participant into EDTA bottles. Plasma samples were separated from the freshly collected blood into Eppendorf tubes by spinning at 3000rpm. The plasma samples extracted were stored at -20°C until they are ready for analysis. A well-structured questionnaire based on demographic characteristics such as age, sex, marital status, occupation, and educational level was used. Hepatitis B and E possible associated risk factors and behavioural characteristics such as the previous history of hepatitis, the source of drinking water, type of toilet, personal hygiene, waste disposal, multiple sexual partners, blood transfusion, interaction with animals, consumption of alcohol and past surgery were recorded. The administered questionnaire was filled by all consenting individuals before sample collection, those who could neither read nor write were assisted.

2.5 Detection of HBsAg

Hepatitis B surface antigens (HBsAg) were screened using enzyme-linked immunosorbent assay (ELISA) kits (Dia.Pro, Milano, Italy) and the other markers of HBV infections was determined using the 5-profile cassette rapid kit (Lumiquick diagnostics, USA). The assay was performed according to the manufacturer's instruction.

2.6 Detection of HEV Antibodies

HEV IgM and IgG antibodies were screened using the serum samples. The test was carried out using AccuDiag™ enzyme-linked immunosorbent assay (ELISA) kits for The ELISA kits were manufactured by Diagnostic Automation Inc., USA. Testing was carried out according to the manufacturer's instructions.

2.7 Data Analysis

Data generated were analyzed using SPSS version 20.0 to compare HBV/HEV positive and negative samples and pier-sons chi-square method were used and the level of significance was set at $P < 0.05$ at 95% confidence interval.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Distribution of HBV sero-markers

Table 1 shows the distribution of HBV sero-makers among the participants. The prevalence of HBsAg was 6.1% (n=11), HBsAb 8.3% (n=15), HBeAg 0.0% (n=0), HBeAb was 26.1% (n=47), and HBcAb 17.8% (n=34).

3.1.2 Overall prevalence of HBV/HEV co-infection

Table 2 shows the overall coinfection rate of HBV and HEV-IgG/IgM antibodies among the participants was 12.2% (n=22/180).

Table 1. Distribution of HBV sero-markers tested for HEV Coinfections

HBV Sero-markers	No. Tested (%)	Positive (%)
HBsAg	180 (100.0)	11 (6.1)
HBsAb	180 (100.0)	15 (8.3)
HBeAg	180 (100.0)	0 (0.0)
HBeAb	180 (100.0)	47 (26.1)
HBcAb	180 (100.0)	32 (17.8)

3.1.3 Prevalence HBV/HEV co-infection among the different study populations

There was low co-infection among the groups, only 1 (0.6%) was positive for HBsAg/HEV IgM and 2 (1.1%) for HBsAg/HEV IgG. HBeAb/HEV IgG coinfection was the highest among butchers, Pig handlers and non-animal handlers. Butchers had the highest prevalence of co-infection (15.9%), followed by pig handlers (14.3%) and then non-animal handlers (7.7%) as shown in Table 2.

3.2 Discussion

Little information has been recorded on HBV/HEV co-infection. A high HBV/HEV co-infection rate (12.2%) was observed in this study. This is comparable to Coursaget et al. [31] who observed a dual acute hepatitis B and E infections in four patients (10.0%) in Chad. Previous studies have reported a lower prevalence of 2.8% by Nim et al. [32] in North India. This could be due to constant sharing of sharp object and regular contact with animal. The observation from this study shows that co-infection of HBV and HEV is high in Nigeria however, none among the cohorts in this study showed any sign of illness which could be attributed to early contact of the disease.

This study shows high prevalence of HBV/HEV co-infection (22.2%) among Butchers and Pig handlers when compared to Non-animal handlers in Osun State, Nigeria. This is lower than 27.3% reported by Ola et al. [33] among healthcare workers in Nigeria. The overall prevalence of HBV/anti-HEV IgG co-infection was 15.8% which is higher than Iran's previous studies found in the general population of the Fars province (13.4%) [34], pregnant women at northern shores of Persian Gulf (6.3%) [23], and blood donors in Tehran (8.1%) [35]; but lower than the value reported among adults in Khuzestan province (46.1%) [22].

The co-infection rate was relatively higher in men (20.1%) than in women (14.1%), but not statistically significant. This finding might be explained by people's lifestyle in the study region, where most men have outdoor jobs while most women stay at home doing housekeeping work. This may contribute to the high level of HEV seropositivity observed in this area. Moreover, the overall co-infection rate of HBV/anti-HEV IgM was 1.6% in our study, giving a low level of recent infection, which is in accordance with previous reports stating 0.5–5.0% HEV incidence among healthy individuals [36].

More significantly, this study stresses the importance of the association of HBV and HEV in a country endemic for hepatitis B, since 12.2% of acute HEV infections were found to be HBsAg positive. Moreover, simultaneous acute hepatitis B and acute HEV infections were detected in four cases (15.0%). This raises the possibility of the presence of coinfection or superinfection by HEV and HBV in a large number of patients. A possible coinfection or superinfection with HEV and HBV or HEV and HAV has been described in two other studies [31,37,38].

Our data analysis also revealed the presence of HEV Ab in one of the HBV/HEV co-infected patients; however, the route of HEV transmission in this individual was unknown. Previous studies reported rare routes of HEV infection transmission through the blood transfusion or sexual contact in addition to the main fecal-orally route [20,39]. Butchers were at higher risk of both HBV/HEV co-infections irrespective of age, sex, marital status and educational status. Extensive exposure to the animals based on occupation could be responsible for the increase in zoonotic HEV infections than in population who are not exposed to contact with animals but may serve as a source of infection to others. There is need for Government intervention in strict control measure, public health programs on these viruses, proper vaccination against HBV, and early diagnosis and treatment should be practiced to prevent further spread. More so, consistent provision of contaminants-free drinking water should be available for consumption.

The limitation of this study was that this study was unable to test for the molecular characterization of HEV. Further studies on HBV and HEV infection is required from different cohorts such as female animal handlers especially the pregnant ones who can transmit the virus to the foetus and also child bearing age to determine the rate at which Animal handlers contribute to the spread of these infections.

Table 2. Prevalence of HBV/HEV co-infection among animal and non-animal handlers

Variables		Butchers (%)	Pig handlers (%)	Non-animal handlers (%)	No. Positive for HBV/HEV coinfection (%)	p - value
HBsAg/HEV IgG	Positive	1(1.4)	1(4.8)	0(0.0)	2(1.1)	0.197
	Negative	68(98.6)	20(95.2)	90(100.0)	178(98.9)	
HBsAb/HEV IgG	Positive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	Negative	69 (100.0)	21 (100.0)	90 (100.0)	180 (100.0)	
HBeAg/HEV IgG	Positive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	Negative	69 (100.0)	21 (100.0)	90 (100.0)	180 (100.0)	
HBeAb/HEV IgG	Positive	8(11.6)	1(4.8)	4(4.4)	13(7.2)	0.329
	Negative	61(88.4)	20(95.2)	86(95.6	167(92.8)	
HBcAb/HEV IgG	Positive	1(1.4)	1(4.8)	3(3.3)	5(2.8)	0.069
	Negative	68(98.6)	20(95.2)	87(96.7)	175(97.2)	
HBsAg/HEV IgM	Positive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	Negative	69 (100.0)	21 (100.0)	90 (100.0)	180 (100.0)	
HBsAb/HEV IgM	Positive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	Negative	69 (100.0)	21 (100.0)	90 (100.0)	180 (100.0)	
HBeAg/HEV IgM	Positive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	Negative	69 (100.0)	21 (100.0)	90 (100.0)	180 (100.0)	
HBeAb/HEV IgM	Positive	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	
	Negative	69 (100.0)	21 (100.0)	90 (100.0)	180 (100.0)	
HBcAb/HEV IgM	Positive	1 (1.4)	0 (0.0)	1 (1.1)	2 (1.1)	0.857
	Negative	68 (98.6)	21 (100.0)	89 (98.9)	178 (98.9)	
Overall HBV and HEV IgG/IgM Coinfection	Positive	11 (16.0)	3 (14.3)	8 (8.9)	22 (12.2)	
	Negative	58 (84.0)	18 (85.7)	82(91.1)	158 (87.8)	
	Total	**69 (100.0)**	**21 (100.0)**	**90 (100.0)**	**180 (100.0)**	

4. CONCLUSION

This study was able to show that there is a high HBV/HEV co-infection rate among animal handlers in Osun State, Nigeria. This can serve as a source for the transmission of these viruses among their family as well as the community. To achieve a full coverage of the general population, the vaccination programs should be extended to all HBsAg/HBsAb negative people. Since the consumption of contaminated water is the main transmission route of HEV, improving the level of public health sanitation in the area should be considered a priority by policymakers.

CONSENT

All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this study.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the Osun State Ministry of Health, Osogbo, Nigeria (OSHREC/PRS/569T/3), and have, therefore, been performed following the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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